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Exchange of Genetic Markers at Extremely High Temperatures in the Archaeon *Sulfolobus acidocaldarius*

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When cells of two auxotrophic mutants of *Sulfolobus acidocaldarius* are mixed and incubated on solid medium, they form stable genetic recombinants which can be selected, enumerated, and characterized. Any of a variety of auxotrophic markers can recombine with each other, and the phenomenon has been observed at temperatures of up to 84°C. The ability to exchange and recombine chromosomal markers appears to be an intrinsic property of *S. acidocaldarius* strains. It occurs between two cell lines derived from the same parent or from different parents and also between a recombinant and its parent. This is the first observation of chromosomal marker exchange in archaea from geothermal environments and provides the first functional evidence of generalized, homologous recombination at such high temperatures.

The upper temperature limit of life has been repeatedly revised in recent years by the isolation of a succession of unusual prokaryotes from solfataras, submarine hot springs, and similar geothermal environments (29). Many of these organisms, termed extreme thermophiles or "hyperthermophiles," grow optimally at temperatures ranging from 80 to 105°C (29). Such growth temperatures imply biochemical mechanisms that prevent or otherwise compensate for in vivo thermal inactivation of proteins, nucleic acids, and other molecules common to all life forms. Hyperthermophiles also have considerable evolutionary significance. Molecular-sequence analyses place them at the deepest branch points of the two prokaryotic domains, i.e., *Bacteria* and *Archaea* (31). Archaea isolated from geothermal environments represent some of the closest known prokaryotic relatives of modern eukaryotes, as judged by sequence comparisons of diverse cellular components. These components include DNA-dependent RNA polymerase (32), translational elongation factors (12), transcriptional promoters (21), soluble transcription factors (11, 23), ATPase subunits (12), aminoacyl tRNA synthetases (4), rRNA-processing enzymes (20), a molecular chaperone (30), and the multicatalytic protease or proteasome (15). Indeed, molecular homology to eukaryotes has made thermophilic archaea useful in elucidating the functions of certain cellular apparatuses of eukaryotes. Analysis of thermophilic archaea, for example, has helped to identify the TRiC complex as a molecular chaperone of the eukaryotic cytosol (28, 30) and to elucidate important structural features of the eukaryotic proteasome (15).

Sulfolobus spp. are aerobic archaea that populate terrestrial hot springs; most grow optimally at about 80°C and pH 3 (6). Nearly all of the genetic phenomena so far described for thermophilic archaea occur in *Sulfolobus* or related species. Various *Sulfolobus* isolates have been found to contain infective viruses (25), transposable genetic elements (26), and at least one self-transmissible conjugative plasmid (24). Site-specific recombination and electrotransfection by purified viral DNA have also been observed (18, 25). However, one of the most fundamental genetic processes, exchange of chromosomal genes, has not been reported for *Sulfolobus* spp. or other thermophilic archaea. In the present study, genetic assays were devised and used to demonstrate that mutant strains of *Sulfolobus acidocaldarius* spontaneously form recombinant phenotypes under appropriate conditions. The results provide the first genetic evidence of chromosomal exchange and recombination in an archaeon from geothermal environments.

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MATERIALS AND METHODS

Strains and growth conditions. Table 1 lists the strains used in this study. *S. acidocaldarius* mutants were isolated by direct selection from unmutagenized cultures (*pyrF* and *eth* mutants) or by brute force screening of UV-mutagenized colonies as previously described (7, 9). The *pyrF42* and *pyrF43* mutations were selected in *S. acidocaldarius* type strain DSM 639, obtained from the American Type Culture Collection, Rockville, Md., as ATCC 33909. All other mutations described in this study were isolated in *S. acidocaldarius* DG6 (ATCC 49426), as summarized in Table 1. These two wild-type strains differ in history (6) and in colony appearance under certain conditions (8).

Media and incubation conditions were essentially those of previous studies (7, 9). Dilution buffer contained the following (in grams per liter of distilled water): K_2SO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; L-glutamic acid, 0.2; gelatin, 0.1. The pH was adjusted to 3.5 with H_2SO_4, and the solution was sterilized by autoclaving. Nonnutritive (MAT) medium contained the following (in grams per liter of distilled water): K_2SO_4, 3.0; NaH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 1.25; $CaCl_2 \cdot 2H_2O$, 0.25; gellan gum (Kelco division of Merck), 6.5; gelatin, 0.1; sufficient H_2SO_4 to yield a final pH of approximately 3.5. Selective medium for enumeration of Pyr^+ (uracil-independent) recombinants contained 0.2% D-xylose, 0.1% L-glutamine, and 0.1% acid-hydrolyzed casein in addition to gelling agent and minerals (7).

A collection of auxotrophic *S. acidocaldarius* mutants (see Results) was qualitatively assayed for genetic exchange as follows. Cells were pelleted by centrifugation and resuspended in dilution buffer. Aliquots containing 3×10^7 cells were then combined with the same number of washed cells of another auxotroph or with a second aliquot of the same auxotroph; the latter case served as the reversion control. Each of the resulting pairwise combinations was plated on minimal medium containing 0.2% D-xylose as the primary carbon source and 0.1% L-glutamine as the nitrogen source. Each test was performed in duplicate, and the resulting prototrophic colonies were counted after 10 days of incubation. Pairs of mutants yielding >20 colonies in combination and ≤1 revertant when plated alone were scored as positive for recombination.

Quantitative assays. To assay recombinant formation under various test conditions, cells were harvested in late exponential phase and resuspended in dilution buffer. A 1:1 mixture containing 10^8 cells of each strain was spread on the surface of a nonnutritive (MAT) plate (6 cm in diameter) and incubated for 20 h at 77°C. Cell number did not increase significantly during this period, as determined by turbidity and viable count, and supplementing the MAT medium with uracil did not significantly affect the frequency of Pyr^+ recombinants recovery. This indicated that the observed frequencies of Pyr^+ recombinants were not biased by preferential growth or survival of recombinants under the test condition.

To enumerate the recombinants formed by the end of this incubation, cells were resuspended from the MAT plates with a sterile glass spreader and dilution

TABLE 1. *S. acidocaldarius* strains used in this study[a]

Designation	Genotype	Phenotype	Source or derivation (reference)
DG6	Wild type	Analog sensitive; prototrophic	Strain C (7)
DG12	*eth-1*	Ethionine resistant	Direct selection (7)
DG29	*pyrD1*	Requires cytosine or uracil	UV mutagenesis (9)
DG33	*rbf-1*	Requires riboflavin	UV mutagenesis
DG38	*cbp-2*	Requires arginine plus cytosine or uracil	UV mutagenesis (9)
DG40	*caa-2*	Requires a combination of amino acids	UV mutagenesis
DG51	*met-1*	Requires methionine	UV mutagenesis
DG55	*his-2*	Requires histidine	UV mutagenesis
DG62	*ilv-2*	Requires isoleucine plus valine	UV mutagenesis
DG64	*pyrB4*	Requires cytosine or uracil	UV mutagenesis (8)
DG66	*pyrF5*	Requires cytosine or uracil	Direct selection[b] (8)
DG97	*eth-4 nov-4 his-2 pyrF10*	Requires cytosine or uracil; novobiocin and ethionine resistant	DG 55 by sequential direct selection (7)
DG180	*pyrF39 eth-4 his-2*	Requires histidine and cytosine or uracil; ethionine resistant	Sequential direct selection[b] (7)
DG200	*pyrF48 cbp-2*	Requires arginine plus cytosine or uracil	Direct selection[b] (9)
DG202	*eth-4 his-2 cbp-2*	Requires histidine plus arginine plus cytosine or uracil; ethionine resistant	DG180 × DG200 (see text)
DG203	*his-2 cbp-2*	Same as DG202 but ethionine sensitive	DG180 × DG200
DG185	Wild type	Analog sensitive; prototrophic	ATCC 33909
DG188	*pyrF42*	Requires cytosine or uracil	Direct selection[b] (9)
DG189	*pyrF43*	Requires cytosine or uracil	Direct selection[b] (9)

[a] All strains are grouped according to the wild-type parent from which they were ultimately derived (i.e., DG6 versus ATCC 33909).
[b] *pyrF* mutants were isolated by spontaneous high-level resistance to 5-fluoroorotate (9).

buffer. Cell densities were determined by photometry (A_{600} in 13-mm-diameter glass tubes was measured with a Milton-Roy Spectronic 21D spectrophotometer) as calibrated with microscopic cell counts and colony formation on complete medium. An aliquot of the resulting suspension, containing approximately 10^7 cells, was plated on selective medium (see above). Pyr^+ colonies were counted after 6 to 8 days of incubation at 77°C, and frequency was expressed as the ratio of the number of colonies to the number of cells plated.

Test conditions were as follows: standard, equal numbers of live cells of each strain; heat-killed cells, boiling of one of the cell suspensions for 10 min in dilution buffer, yielding less than two viable cells per mating; culture supernatant, 50 μl of twice-centrifuged (10 min each time at approximately 10,000 × *g*) cell-free culture medium substituted for the cell suspension of one of the strains; purified DNA, 6 μg of genomic DNA of a prototrophic *S. acidocaldarius* strain (DG12) added to the cells. Tests at temperatures above 77°C were performed in sealed glass jars submerged in constant-temperature water baths. Reversion frequencies were measured by plating multiple, independent cultures of the appropriate auxotroph on selective medium. The number of prototrophic colonies that formed was divided by the number of cells plated.

The above-described two-stage procedure had been empirically found to yield efficient recombinant formation in the first stage (MAT plate) but not in the second stage (selective plate). In addition, the actual number of Pyr^+ colonies forming on the selective plate itself (late, i.e., residual, recombination) could be estimated by a separate control in which the two strains were plated side by side on the MAT plate. The cells were thus prevented from making contact during the test itself but were resuspended and plated together on the selective medium in the same way as the experimental samples.

The unselected markers in crosses of strains DG64 and DG180 were scored as follows. Pyr^+ recombinants were selected as described above but with additional histidine (40 μg/ml) in the plates to minimize preferential recovery of His^+ over His^- colonies. Isolated colonies were then picked with sterile applicator sticks and transferred in a grid pattern to fresh plates containing (i) the same medium, (ii) the same medium supplemented with 200 μg of L-ethionine per ml, and (iii) xylose-glutamine medium lacking all other amino acids. Growth of the resulting patches was scored after 3 to 4 days of incubation.

Orotidine 5′-monophosphate decarboxylase activity was assayed spectrophotometrically in dialyzed cell extracts of strains DG66 (*pyrF5*) and DG97 (*pyrF10*) at 70°C as previously described (9). The specific activity was found to be less than about 10% of wild-type levels in both strains, in agreement with the growth phenotype of these mutants (9).

RESULTS AND DISCUSSION

Formation of prototrophic recombinants. A variety of auxotrophic mutants of the archaeon *Sulfolobus acidocaldarius* have been isolated by using standard techniques of microbial genetics (7, 9); a subset of this collection is listed in Table 1. During characterization of these mutants, it was fortuitously observed that mixtures of two stable auxotrophs plated on minimal medium yielded many prototrophic colonies. This suggested that a form of chromosomal gene transfer and recombination was occurring on the plates of minimal medium. The phenomenon was tested in a systematic fashion and found to be a general property of the available *S. acidocaldarius* auxotrophs. In qualitative assays (see Materials and Methods), each of the DG6 derivatives DG29, DG33, DG38, DG40, DG51, DG55, DG62, DG64, and DG66 scored positive in all pairwise combinations with all other members of the group. In separate experiments (see below), recombination between the *eth-4* and *his-2* mutations was also observed. Thus, a total of at least 10 distinct genetic loci defined by the corresponding mutations (Table 1) were observed to recombine. The phenomenon was not restricted to *S. acidocaldarius* strains derived from DG6; it occurred also between auxotrophic derivatives of ATCC 33909 (Table 2) and between DG6 derivatives and ATCC 33909 derivatives (Table 3).

Under normal assay conditions, prototrophs formed at a frequency of about 10^{-5} per cell plated. This frequency is 100 to 1,000 times the spontaneous reversion frequencies of the mutants employed and about 30 times the mutation frequencies typically induced by chemical or UV light mutagenesis of *S. acidocaldarius* (7). Explanations, other than genetic recombination, for the high yield of prototrophic clones were experimentally tested. For example, the possibility was tested that auxotrophs yielded more revertants when plated in mixtures than when plated alone because of cross-feeding on the selective medium. (The ensuing residual growth could conceivably support additional reversion, either by normal or by directed ["Cairnsian"] mechanisms [5].) This question was addressed in two ways. (i) In qualitative tests of fertility among auxotrophs with different requirements, reversion controls were plated on the same plate immediately adjacent to cell mixtures to promote cross-feeding of the reversion control. Among the nine auxotrophs (see above) tested in this manner (about 3×10^7 cells in each case), only one revertant colony was observed. (ii) Pairs of *pyrF* mutants were assayed for prototroph formation. These mutants lack the last two enzymes of de novo UMP biosynthesis (9). Typical results are shown in Fig. 1 and Table

TABLE 2. Effects of various test conditions on recombinant formation

Condition[a]	Avg no. of Pyr^+ colonies/10^6 cells[b] (SD)	No. of independent trials[c]
DG64 + DG180[d]		
Standard	14.6 (3.9)	7
Live + heat-killed cells[e]	<0.3 (0.25)	6
Cells + culture supernatant[e]	<0.12 (0.04)	5
Cells + purified *pyr*$^+$ DNA[e]	<0.18 (0.02)	5
Late recombination	0.54 (0.09)	19
DG188 + DG189[f]		
79°C	7.5 (4.7)	3
80°C	3.3 (0.9)	3
81°C	5.6 (1.1)	2
82°C	7.3 (5.7)	4
84°C	6.2 (1.9)	3
Late recombination	0.18 (0.08)	9

[a] Recombination frequencies were determined by the modified assay of Fig. 2; the individual test conditions are described in Materials and Methods.
[b] Values have not been corrected for late (residual) recombination, frequencies of which are listed for comparison.
[c] Number of independent determinations (performed on different days with different cultures of the indicated strains) averaged to yield the frequency shown.
[d] Frequencies of DG64 and DG180 reversion to Pyr^+ were 0.03 and $0.02/10^6$ cells, respectively.
[e] In cases involving only one live culture, the results include at least two trials with each strain. For each of these treatments, a standard test in parallel, with the same cultures and media, yielded recombinants.
[f] Frequencies of DG188 and DG189 reversion to Pyr^+ were less than 0.03 and $0.02/10^6$ cells, respectively.

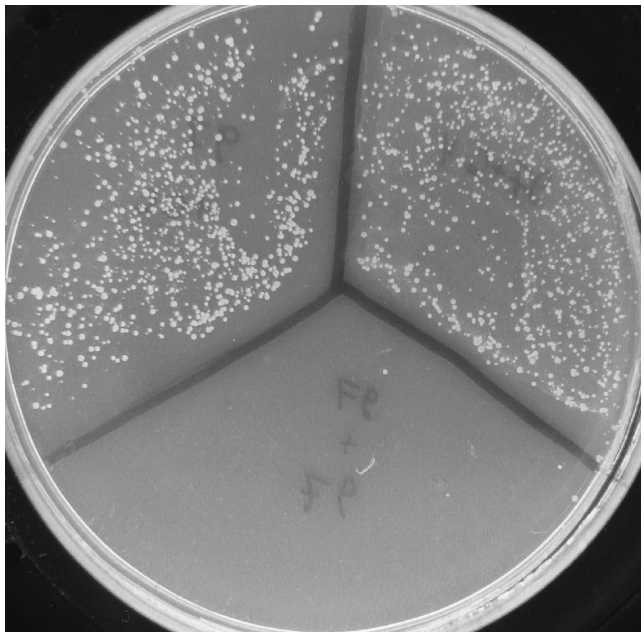

FIG. 1. Formation of prototrophic *S. acidocaldarius* clones from auxotrophic mixtures. Upper left, prototrophic colonies resulting from a mixture of 10^7 cells each of DG66 and DG97; upper right, prototrophic colonies resulting from a mixture of DG97 and DG64 cells; lower center, four phenotypic revertants resulting from 2×10^7 DG97 cells plated alone. Strains DG64 and DG66 yielded 0 and 16 revertants, respectively, from 2×10^7 cells plated adjacent to cell mixtures in this same manner (data not shown).

2. *pyrF* mutants DG66 and DG97, for example, formed prototrophs efficiently, despite an intrinsic inability to be cross-fed. Reversion controls on the same plates as cell mixtures yielded few or no colonies (Fig. 1).

Analysis of unselected markers. Another possible explanation for the observed prototrophic colonies was the formation of heterozygous diploids, i.e., genetic complementation (10). To distinguish this possibility from the formation of genetic recombinants, crosses were performed with strains having a total of four genetic markers (Table 4). Only two of four available markers were used to select a recombinant phenotype, and the remaining (i.e., unselected) two markers were then scored among the resulting colonies. Since the colonies were scored under continued selection for the Pyr^+ phenotype (see Materials and Methods), heterozygous diploids should have been stabilized, yielding only the dominant phenotype for each unselected marker (His^+, e.g.). This was not the experimental result (Table 4); both the His^+ and His^- phenotypes and both the Eth^r and Eth^s phenotypes were found at reasonable frequencies. Furthermore, these markers segregated independently of each other (Table 4).

In addition to heterozygosity, the data of Table 4 are incompatible with reversion as the sole source of colony formation. The nonparental combinations of unselected markers (ethionine-resistant prototrophs, e.g.) occurred at frequencies above about 10^{-6} per cell plated. Generation of this phenotypic combination from either parent by reversion requires two

TABLE 3. Fertility of various strain combinations

Phenotype selected	Reversion control[a]		Mixtures[b]	
	Strain	No. of colonies	Strains	No. of colonies
DG6 derived × ATCC 33909 derived				
Pyr^+	DG64	0	DG64 × DG180	170
Pyr^+	DG180	1	DG64 × DG188	508
Pyr^+	DG188	0	DG64 × DG189	425
Pyr^+	DG189	0	DG180 × DG188	265
			DG180 × DG189	240
			DG188 × DG189	419
Recombinant × parent				
Pyr^+	DG180	0	DG180 × DG202	1,156
Pyr^+	DG202	8	DG180 × DG203	287
Pyr^+	DG203	0		
$PyrF^+$ His^+	DG200	0	DG200 × DG202	720
$PyrF^+$ His^+	DG202	0	DG200 × DG203	1,040
$PyrF^+$	DG203	0		

[a] Approximately 6×10^7 cells of a homogeneous culture were plated.
[b] Approximately 3×10^7 cells of each strain were plated as a mixture.

TABLE 4. Independent segregation of *S. acidocaldarius* markers in genetic exchange[a]

Trial no.	No. of Pyr^+ colonies scored[b]	% of Pyr^+ colonies with phenotype[c] of:				Nonparental total
		His^+ Eth^s	His^- Eth^r	His^+ Eth^r	His^- Eth^s	
1	88	38.6	39.8	10.2	11.4	21.6
2	184	55.4	21.2	16.3	7.1	23.4
3	166	34.3	44.0	7.2	14.5	21.7
4	185	47.0	28.6	12.4	11.9	24.3

[a] Parental strains were DG64 and DG180.
[b] Colonies that formed on plates lacking uracil but containing histidine were individually tested for growth in the presence of ethionine (200 μg/ml) or in the absence of histidine.
[c] Frequencies of Pyr^+ His^+ Eth^r class formation by spontaneous mutation were predicted from the observed individual reversion frequencies (see Materials and Methods) as follows: for DG64, $(3 \times 10^{-8})(2 \times 10^{-9}) = 6 \times 10^{-17}$ per cell plated; for DG180, $(1 \times 10^{-8})(4 \times 10^{-9}) = 4 \times 10^{-17}$ per cell plated.

independent mutations. At the observed frequencies of single revertants, these would occur at an expected frequency of about 10^{-16} per cell plated, which is a factor of about 10^9 below the detection limit of the experiment. The fact that the recombinants were rather evenly distributed among all four possible phenotypic classes (Table 4) indicates that recombination events between the corresponding genes on the *Sulfolobus* chromosome, which is a single, circular DNA molecule (13), were frequent.

Mechanistic aspects. The above results indicated that for pairs of sufficiently stable auxotrophs, most of the colonies which form under these conditions are various genetic recombinants of the two original *S. acidocaldarius* strains. To investigate the basic mechanism of this marker exchange process, the basic assay was modified so that various conditions could be tested for their effects on recombinant formation without requiring a corresponding change in conditions for enumeration of recombinants (Fig. 2). An additional benefit of this modified assay was that the contribution of late (i.e., residual) exchange on the selective plate itself was minimized and could be directly estimated with a simple control (Fig. 2).

Among a variety of conditions tested in this manner, three were found to effectively abolish genetic exchange (Table 2): (i) severe heating of either of the two cell suspensions before mixing, (ii) substitution of culture supernatant for either of the two cell suspensions, and (iii) substitution of purified chromosomal DNA for either of the two cell suspensions. Conversely, genetic exchange was not impaired by increasing the test temperature to up to 84°C or washing the cells free of culture supernatant before mixing (the standard assay; Table 2).

The available data portray the exchange of *S. acidocaldarius* markers as a cell mediated, but symmetrical, process. On average, each unselected allele of two *Sulfolobus* parents occurred with the same frequency among recombinants (Table 4) and no evidence of obligate donor-recipient relationships among the various strains tested in this study was found. For example, two parental strains could be nearly isogenic, each having been derived from a common parent in a single step, and still exchange genes efficiently (Fig. 1 and Table 2). Furthermore, strains derived from a genetic cross scored positive in recombination tests with their parents (Table 3). Finally, it seems that many regions of the chromosome can be exchanged, since it is unlikely that all 10 of the metabolically distinct genes observed to recombine (see above) are confined to one small interval of the *S. acidocaldarius* chromosome.

Biological significance. This is the first report of chromosomal gene exchange in *Sulfolobus* spp. and provides the first functional evidence of generalized homologous recombination in an archaeon that populates geothermal environments. Conjugational self-transmission of a large plasmid among various *Sulfolobus* isolates has also been recently demonstrated, and its cellular mechanism has been characterized (24). Transfer of a 45-kb plasmid, designated pNOB8, occurred spontaneously and efficiently in liquid cultures; donor and recipient cells aggregated upon mixing and formed intercellular connections observed by electron microscopy (24). The subsequent rate of transfer of pNOB8 was high, despite agitation of liquid cultures (24). Plasmid DNA has not been observed in authentic *S. acidocaldarius* strains, either by gel electrophoresis of restriction fragments (6, 8) or by buoyant-density centrifugation of total DNA (8). In addition, *S. acidocaldarius* did not receive and propagate pNOB8 under conditions promoting transfer and propagation of this plasmid in other *Sulfolobus* isolates (24). The available data thus suggest that these two forms of genetic exchange observed in *Sulfolobus* spp. are mechanistically distinct.

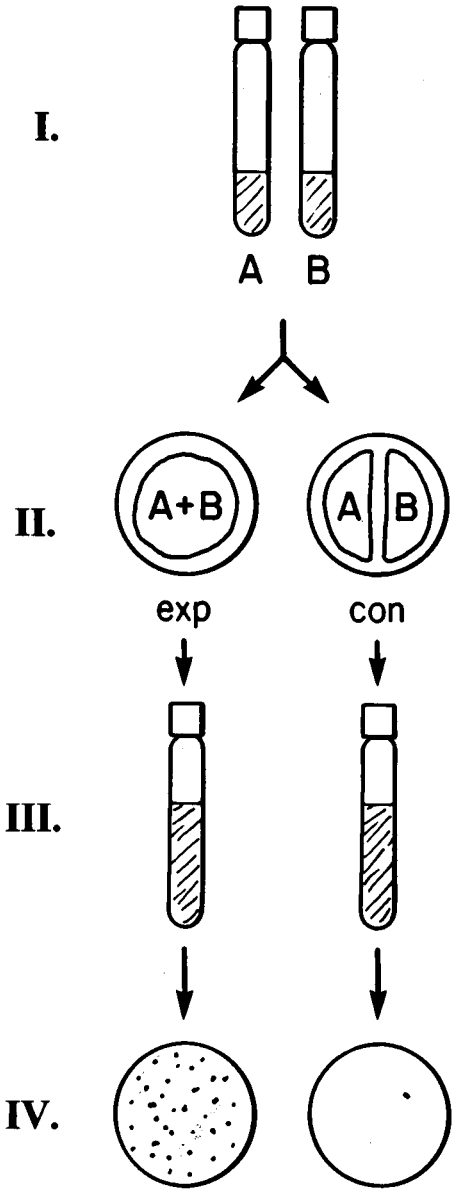

FIG. 2. Quantitative assay for recombinant formation under various conditions. (I) Cells of two auxotrophic mutants (A and B) were suspended in dilution buffer. (II) Aliquots (10^8 cells) of each strain were deposited on plates of nonnutritive medium and incubated for 20 h at 77°C. For the late-recombination control (con), cells of the two auxotrophs remained separate during this first incubation but were otherwise processed identically to the experimental (exp) mixtures. (III) The nonnutritive plates were allowed to cool, and the cells were eluted in buffer. (IV) Aliquots of the resulting cell suspensions were plated on selective nutritive medium (lacking uracil, e.g.) and incubated for 6 to 8 days at 77°C to allow formation of prototrophic colonies. See Materials and Methods for additional experimental details.

Chromosomal marker exchange in *S. acidocaldarius* may not conform to the model of bacterial conjugation. Although cell-cell contact seems to be required, a donor-recipient relationship cannot be inferred and all strains seem generally fertile with each other. These nonbacterial properties have a precedent in the mating system of another archaeon, however: *Haloferax volcanii* (17, 22). The morphology and cell envelope architecture of *H. volcanii*, a mesophilic extreme halophile, are unique to archaea and similar to those of *S. acidocaldarius* (2). *H. volcanii* cells can spontaneously form cytoplasmic bridges

and cell fusions (22), which may explain the observed properties of its genetic exchange (17, 22). Efforts to understand the exchange process of *S. acidocaldarius* similarly in cellular terms are under way.

The existence of spontaneous chromosomal exchange in *S. acidocaldarius* raises basic questions relevant to the evolution of primitive sexual processes and their significance for life in geothermal environments. It implies, for example, that *S. acidocaldarius* populations may have a partial sexual character, i.e., that chromosomal mutations which arise in one cell lineage can be transferred to other lineages. This possibility has implications for the evolutionary dynamics of *Sulfolobus* spp. in nature, since lateral gene flow provides genetic diversity and evolutionary adaptability not available to strictly asexual (i.e., clonal) microbial populations (27). The fact that archaea isolated from various thermal habitats possess temperate viruses, transposable genetic elements, and complex plasmids capable of interspecific transfer (24–26, 33) further underscores the potential for genetic flux within geothermal environments. These habitats can harbor unexpectedly complex communities of thermophilic archaea (1); they also may represent one of the closest modern approximations to the geochemical and biological context in which cells evolved (19).

It should also be considered that *S. acidocaldarius* may benefit from chromosomal exchange as a mechanism of genomic repair. As demonstrated in Table 4, *S. acidocaldarius* can spontaneously assemble selectable, but otherwise rare, combinations of alleles from two multiply mutated strains. In nature, this same mechanism may allow two *S. acidocaldarius* cells with deleterious DNA lesions to recombine and thus reconstruct a single, viable genome. Conjugative DNA repair is believed to have been an important survival mechanism for primordial, haploid microorganisms subjected to intense UV radiation on the early earth (3, 16). An analogous survival advantage for *S. acidocaldarius* in its modern geothermal habitats seems plausible, on the basis of the known damaging effects of oxygen and high temperature on the primary structure of DNA (14).

Finally, on a practical level, the exchange mechanism opens up new experimental approaches to the study of genetic processes at extremely high temperature. Recombinational events can be enumerated by genetic assay, facilitating study of the exchange and recombination processes in vivo. Also, by first selecting and then mating *pyrF* derivatives of two auxotrophs, it has been possible to combine two preexisting, nonselectable mutations in a single strain. This technique, demonstrated by the isolation of strains DG202 and DG203 (Table 1), represents one of the first general methods of strain construction for archaea from geothermal environments.

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REFERENCES

1. **Barnes, S. M., R. Fundyga, M. Jeffries, and N. Pace.** 1994. Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. Proc. Natl. Acad. Sci. USA **91:**1609–1613.
2. **Baumeister, W., I. Wildhaber, and B. Phipps.** 1989. Principles of organization in eubacterial and archaebacterial surface proteins. Can. J. Microbiol. **35:**215–227.
3. **Bernstein, H., H. Byerly, F. Hopf, and R. Michod.** 1985. DNA repair and complementation: the major factors in the origin and maintenance of sex, p. 29–45. *In* H. Halvorson, and A. Monroy (ed.), The origin and evolution of sex. Alan R. Liss, Inc., New York.
4. **Brown, J. R., and W. F. Doolittle.** 1995. Root of the universal tree of life based on ancient aminoacyl-tRNA synthetase gene duplications. Proc. Natl. Acad. Sci. USA **92:**2441–2445.
5. **Foster, P.** 1992. Directed mutation: between unicorns and goats. J. Bacteriol. **174:**1711–1716.
6. **Grogan, D. W.** 1989. Phenotypic characterization of the archaebacterial genus *Sulfolobus*: comparison of five wild-type strains. J. Bacteriol. **171:**6710–6719.
7. **Grogan, D. W.** 1991. Selectable mutant phenotypes of the extremely thermophilic archaebacterium *Sulfolobus acidocaldarius.* J. Bacteriol. **173:**7725–7727.
8. **Grogan, D. W.** Unpublished data.
9. **Grogan, D. W., and R. P. Gunsalus.** 1993. *Sulfolobus acidocaldarius* synthesizes UMP via a standard de novo pathway: results of a biochemical-genetic study. J. Bacteriol. **175:**1500–1507.
10. **Hopwood, D. A.** 1981. Genetic studies with bacterial protoplasts. Annu. Rev. Microbiol. **35:**237–272.
11. **Hüdepohl, U., W.-D. Reiter, and W. Zillig.** 1992. In vitro transcription of two rRNA genes of the archaebacterium *Sulfolobus* sp. B12 indicates a cofactor requirement for specific initiation. Proc. Natl. Acad. Sci. USA **87:**5851–5855.
12. **Iwabe, N., K.-I. Kuma, M. Hasegawa, S. Osawa, and T. Miyata.** 1989. Evolutionary relationship of archaebacteria, eubacteria, and eukaryotes inferred from phylogenetic trees of duplicated genes. Proc. Natl. Acad. Sci. USA **86:**9355–9359.
13. **Kondo, S., A. Yamagishi, and T. Oshima.** 1993. A physical map of the sulfur-dependent archaebacterium *Sulfolobus acidocaldarius* 7 chromosome. J. Bacteriol. **175:**1532–1536.
14. **Lindahl, T.** 1993. Instability and decay of the primary structure of DNA. Nature (London) **362:**709–715.
15. **Löwe, J., D. Stock, B. Jap, P. Zwickl, W. Baumeister, and R. Huber.** 1995. Crystal structure of the 20S proteasome from the archaeon *T. acidophilum* at 3.4 Å resolution. Science **268:**533–540.
16. **Margulis, L., D. Sagan, and L. Olendzenski.** 1985. What is sex? p. 69–85. *In* H. Halvorson, and A. Monroy (ed.), The origin and evolution of sex. Alan R. Liss, Inc., New York.
17. **Mevarech, M., and R. Werczberger.** 1985. Genetic transfer in *Halobacterium volcanii*. J. Bacteriol. **162:**461–462.
18. **Muskhelishvili, G., P. Palm, and W. Zillig.** 1993. SSV1-encoded site-specific recombination system in *Sulfolobus shibatae*. Mol. Gen. Genet. **237:**334–342.
19. **Pace, N. R.** 1991. Origin of life: facing up to the physical setting. Cell **65:** 531–533.
20. **Potter, S., P. Durovic, and P. P. Dennis.** 1995. Ribosomal RNA precursor processing by a eukaryotic U3 small nucleolar RNA-like molecule in an archaeon. Science **268:**1056–1060.
21. **Reiter, W.-D., P. Palm, and W. Zillig.** 1988. Analysis of transcription in the archaebacterium *Sulfolobus* indicates that archaebacterial promoters are homologous to eukaryotic PolII promoters. Nucleic Acids Res. **16:**1–19.
22. **Rosenshein, I., R. Tchelet, and M. Mevarech.** 1989. The mechanism of DNA transfer in the mating system of an archaebacterium. Science **245:**1387–1389.
23. **Rowlands, T., P. Baumann, and S. Jackson.** 1994. The TATA-binding protein: a general transcription factor in eukaryotes and archaebacteria. Science **264:**1326–1329.
24. **Schleper, C., I. Holz, D. Janekovic, J. Murphy, and W. Zillig.** 1995. A multicopy plasmid of the extremely thermophilic archaeon *Sulfolobus* effects its transfer to recipients by mating. J. Bacteriol. **177:**4417–4426.
25. **Schleper, C., K. Kubo, and W. Zillig.** 1992. The particle SSV1 from the extremely thermophilic archaeon *Sulfolobus* is a virus: demonstration of infectivity and of transfection with viral DNA. Proc. Natl. Acad. Sci. USA **89:** 7645–7649.
26. **Schleper, C., R. Röder, T. Singer, and W. Zillig.** 1994. An insertion element of the extremely thermophilic archaeon *Sulfolobus acidocaldarius* transposes into the endogenous β-galactosidase gene. Mol. Gen. Genet. **243:**91–96.
27. **Smith, J. M.** 1978. The evolution of sex, p. 11–32. Cambridge University Press.
28. **Sternlicht, H., G. W. Farr, M. L. Sternlicht, J. K. Driscoll, K. Willison, and M. B. Jaffe.** 1993. The t-complex polypeptide 1 complex is a chaperonin for tubulin and actin *in vivo*. Proc. Natl. Acad. Sci. USA **90:**9422–9426.
29. **Stetter, K. O., G. Fiala, R. Huber, and A. Segerer.** 1990. Hyperthermophilic microorganisms. FEMS Microbiol. Rev. **75:**117–124.
30. **Trent, J. D., E. Nimmesgern, J. Wall, F.-U. Hartl, and A. Horwich.** 1991. A molecular chaperone from a thermophilic archaebacterium is related to the eukaryotic protein t-complex polypeptide-1. Nature (London) **354:**490–493.
31. **Woese, C. R., O. Kandler, and M. Wheelis.** 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc. Natl. Acad. Sci. USA **87:**4576–4579.
32. **Zillig, W., H.-P. Klenk, P. Palm, G. Pühler, F. Gropp, R. Garrett, and H. Leffers.** 1989. The phylogenetic relations of DNA-dependent RNA polymerases of archaebacteria, eukaryotes, and eubacteria. Can. J. Microbiol. **35:**73–80.
33. **Zillig, W., A. Kletzin, C. Schleper, I. Holz, D. Janekovic, J. Hain, M. Lanzendörfer, and J. K. Kristiansson.** 1994. Screening for *Sulfolobales*, their plasmids and their viruses in Icelandic solfataras. Syst. Appl. Microbiol. **16:**609–628.